networks, such as that of MYC, remains to be determined. While Rui et al. (2010) showed that JAK2 inhibitors affect chromatin in PMBL and abrogate an oncogenic program, whether these agents affect MPN in the same manner remains to be determined. On the horizon are histone demethylase inhibitors. A JMJDC2 inhibitor was recently identified (Hamada et al., 2010), and it will be critical to test the activity of such agents in PMBL, HL, MPN, and other tumors that harbor the 9p23-24 amplicon.

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New Antibody to Stop Tumor Angiogenesis and Lymphatic Spread by Blocking Receptor Partnering

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DOI 10.1016/j.ccr.2010.11.030

Tvorogov et al. (2010) describe in this issue of *Cancer Cell* an antibody that inhibits homodimerization of vascular endothelial growth factor receptor-3 (VEGFR-3) and its heterodimerization with VEGFR-2, but not ligand binding. The work provides mechanistic insights into receptor dimerization and an approach to suppress both angiogenesis and lymphangiogenesis.

More than 800,000 cancer patients worldwide are currently being treated with angiogenesis inhibitors. Treatment with the monoclonal antibody bevacizumab to block vascular endothelial growth factor (VEGF), a cytokine that promotes blood vessel growth, delays progression, and prolongs survival in some cancers (Bagri et al., 2010). Other macromolecular therapeutics that block VEGF signaling, including ramucirumab, an antibody that targets VEGF receptor-2 (VEGFR-2), and aflibercept, a chimeric decoy receptor that binds VEGF, are in advanced clinical trials (http://www.clinicaltrials.gov). These agents are selective, are well tolerated, and generally have only modest side effects restricted to consequences of inhibiting VEGF in normal organs.

However, selective VEGF blockers are efficacious in many cancers only when administered in combination with chemotherapy, and tumors can progress while on therapy. The slowing of tumor growth after inhibition of VEGF signaling can be accompanied by increased invasiveness and metastasis in some preclinical models (Paez-Ribes et al., 2009). The mechanisms of dependence on chemotherapy, progression during treatment, and exaggerated aggressiveness are unclear, but more efficacious approaches are actively being sought.

Receptor-blocking antibodies that target the ligand-binding site of receptors compete with the ligand. This type of inhibitor has the potential limitation of being less efficient at high ligand concentrations, when the ligand out-competes the inhibitor. Because delivery of antibodies to tumors is hampered by inefficient blood vessels, erratic blood flow, and high intratumoral pressure, inhibitors may not reach their molecular targets in sufficient amount and uniformity to be fully efficacious. In addition, other mechanisms contribute to the limitations of efficacy of angiogenesis inhibitors. Factors other than VEGF can promote

angiogenesis in tumors, and invading tumors can co-opt normal blood vessels. Even when VEGF blockade slows tumor angiogenesis, it does not slow the growth of lymphatic vessels (lymphangiogenesis) that serve as routes for cancer cells to spread to lymph nodes and distant sites. Because lymphatic metastases have detrimental consequences, selective inhibitors of lymphangiogenesis would complement angiogenesis inhibitors, but none is yet available for clinical use.

One strategy for increasing efficacy is to block the spread of tumor cells to local lymph nodes. Lymphangiogenesis is driven by VEGF-C and VEGF-D, which signal through VEGFR-3. VEGF-C also promotes the formation of VEGFR-2/VEGFR-3 heterodimers. Like VEGFR-2, VEGFR-3 signaling can contribute to angiogenesis in tumors, in which the receptor is expressed on tumor blood vessels as well as on lymphatics. Involvement of VEGFR-3 in the growth of blood vessels and lymphatics makes it a promising candidate for cancer therapy. Inhibition of lymphangiogenesis with a soluble form of VEGFR-3 or monoclonal antibodies that block receptor activation can reduce lymphatic metastases by 50%–70% in preclinical models (Tammela and Alitalo, 2010). Monoclonal antibodies that block binding of VEGF-C and VEGF-D to VEGFR-3 can also suppress angiogenesis, and this action is strengthened when used in combination with a VEGFR-2-blocking antibody (Tammela et al., 2008).

Findings in a report in this issue of *Cancer Cell* (Tvorogov et al., 2010) indicate that efficacy of angiogenesis inhibitors may be increased through use of a combination of two distinct classes of antibodies directed toward functionally different regions of VEGF receptors (Figure 1). Tvorogov and colleagues describe a novel type of VEGFR-3-blocking antibody that inhibits the dimerization of VEGFR-3, which is an essential step in receptor activation. Ligand binding causes VEGF tyrosine kinase receptors to dimerize and become activated through transphosphorylation (Lemmon and Schlessinger, 2010). Because VEGF ligands are dimers, they can trigger monomers of VEGFR-2 and VEGFR-3 to bind one another to form homodimers or heterodimers.

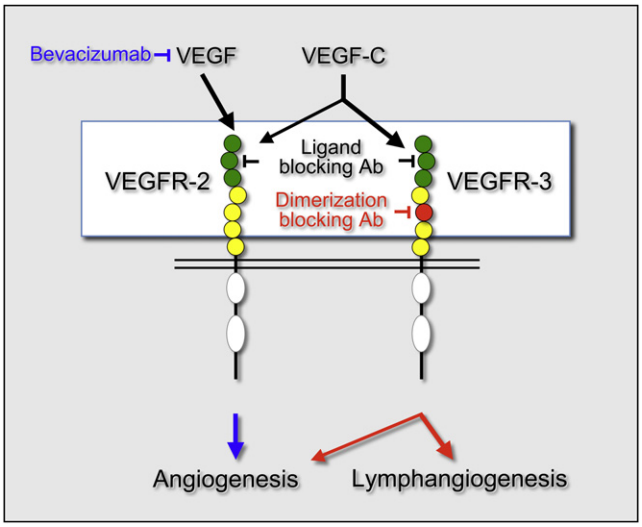

Figure 1. Novel Strategy to Stop Angiogenesis and Lymphatic Growth by Blocking Receptor Dimerization

VEGF binds to and activates VEGFR-2 on the cell surface, which promotes angiogenesis. The antiligand antibody bevacizumab blocks VEGF. VEGF-C, the VEGFR-3 ligand, can also bind to VEGFR-2, and promote receptor heterodimerization, downstream signaling, and endothelial cell sprouting. Conventional inhibitory antibodies (Ab) to VEGFR-2 or VEGFR-3 block ligand binding to extracellular domains 2 and 3. The report by Tvorogov and colleagues describes a novel antibody 2E11 that is directed against the VEGFR-3 extracellular domain 5 that inhibits VEGFR-3 homodimerization and VEGFR-2/VEGFR-3 heterodimerization without blocking ligand binding. This inhibition impairs both angiogenesis and lymphangiogenesis.

Findings by Tvorogov et al. show that the novel antibody 2E11 inhibits the formation of VEGFR-3 homodimers and VEGFR-3/VEGFR-2 heterodimers but does not inhibit binding of VEGF-C ligand to VEGFR-3, unlike conventional receptor-blocking antibodies (Figure 1). The activity of 2E11 was thus relatively independent of ligand concentration. Indeed, the antibody was able to suppress VEGFR-3 activation at even higher concentrations of VEGF-C than those that occur in tissues. Further experiments revealed that the antibody binds to the immunoglobulin-like domain 5 in the extracellular part of VEGFR-3, which is not involved in ligand binding. In VEGF receptors, domains 2 and 3 contribute solely to ligand-binding, but dimerization involves the membrane proximal domain 7 (Lemmon and Schlessinger, 2010; Leppanen et al., 2010). Additional homotypic interactions occur around domain 4 in this class of receptor, suggesting that 2E11 targeting of domain 5 disrupts these interactions (Ruch et al., 2007).

Functional studies showed that the dimerization-blocking action of antibody 2E11 was accompanied by inhibition of VEGF-C-induced phosphorylation of VEGFR-3 and suppression of signal transduction and endothelial cell migration and sprouting. The antibody also suppressed vascular network formation from human endothelial cells implanted in mice. Importantly, the receptor dimerization-blocking antibody used in combination with a ligand-binding antibody produced greater inhibition of VEGFR-3, had synergistic inhibitory effects in some models, and inhibited endothelial sprouting and angiogenic vascular network formation more than either antibody used alone.

Therapeutic antibodies that target receptors can act through multiple mechanisms. Both clinically approved antibodies (cetuximab and panitumumab) that target EGFR (ErbB1, HER1) compete with EGF ligand by binding to domain 3 of the extracellular part of EGFR. Successful interaction blocks ligand binding and receptor activation. The antibody trastuzumab acts on HER2 (ErbB2) through a mechanism not involving inhibition of ligand binding, given that no ligand for HER2 has been identified and homodimerization does not occur. Although the mechanism is not completely understood, trastuzumab has little effect on heterodimerization. By contrast, another HER2 blocking antibody, pertuzumab, acts by blocking heterodimerization of HER2 with HER3 (ErbB3) or another EGFR family member (Hughes et al., 2009). This difference in mechanism of action explains why pertuzumab is effective in carcinomas that express low levels of HER2, where trastuzumab is not. Combinations of the two antibodies produce more potent inhibition of proliferation and migration of tumor cells and induce clustering of crosslinked receptors without receptor activation, followed by inhibition of receptor recycling

and receptor downregulation (Spangler et al., 2010). Antibody combinations are now being tested in HER2-positive breast cancer (http://www.clinicaltrials.gov).

Overall, the findings reported by Tvorogov and colleagues define a novel class of anti-VEGFR-3 antibody that blocks homodimerization and heterodimerization of receptors and complements the activity of antibodies that block ligand-binding. This work provides mechanistic insights into receptor dimerization and the promise of using inhibitors of dimerization as a biologically meaningful approach for suppressing angiogenesis and lymphangiogenesis and potentially tumor growth and dissemination. Because VEGFR-3 is one of the most highly upregulated therapeutic targets in endothelial cells of tumor vessels, the receptor could also serve as a target for antibodies coupled to therapeutic cargo such as radioisotopes, liposomes, or nanoparticles loaded with cytotoxic therapeutics, or even T cells.

This novel class of inhibitors has the potential of outperforming conventional competitive inhibitors of angiogenesis because of the insensitivity to ligand concentration and the ability to inhibit heterodimerization and influence multiple downstream signaling pathways. The use of an antidimerization antibody in combination with an antiligand binding antibody could translate into clinical benefit from more potent antiangiogenic and antilymphangiogenic activities. Further validation of the efficacy of antibody combinations in preclinical models could pave the way for inhibitors that block tumor angiogenesis and lymph node metastasis in cancer patients.

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The Ids Have It

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DOI 10.1016/j.ccr.2010.11.028

In this issue of *Cancer Cell,* Anido et al. demonstrate that Id1 is the likely arbiter of divergent transforming growth factor-β (TGF-β) signaling in glioma-initiating cells (GICs) from different tumors. These findings hold both the promise and potential peril of therapeutic targeting of the TGF-β pathway.

Human glioblastoma derived GICs have stem cell properties of self-renewal and differentiation with genotypes and phenotypes similar to their parental tumors and substantially different from conventional glioma cell lines (Lee et al., 2006). In addition, there is evidence suggesting that GICs can promote tumor angiogenesis and mediate radiotherapy resistance (Bao et al., 2006). With the increasing evidence that GICs are the stem cell subcomponent of malignant gliomas, there are reasons to believe that targeting GICs hold great therapeutic potential. Terminal differentiation is a powerful tumor suppressor mechanism, and thus there is keen interest in finding ways to activate cancer stem cell differentiation programs for therapeutic purposes.

As the founding member of a group of more than 40 secreted factor family members, TGF-β plays an intricate role in the regulation of almost all cell types in the body, with an emphasis on controlling homeostasis and developmental processes including stem cell differentiation. The effects of TGF-β signaling are mediated through transmembrane serine-threonine type I (TβRI) and type II (TβRII) receptors that phosphorylate Smad proteins, which then forward signals from the TGF-β receptors to the nucleus where they regulate transcription. The role of TGFβ in cancer stem cell differentiation is of significant interest given its overexpression in a number of tumors including lung, colon, and gastric carcinoma as well as in high-grade gliomas. TGF-β had been previously shown to increase the self-renewal and oncogenic potential of GICs, although the mechanism was not known (Penuelas et al.,